reconstituted system, we captured structural correlates that precede Bax homo-oligomerization elucidating previously inaccessible steps of the core molecular mechanism by which Bcl-2 family proteins regulate membrane permeabilization. The three-dimensional reconstructions of these membrane assemblies demonstrate that in the presence of a BID BH3 peptide, an individual BAX molecule can insert into the nanodisc membrane, distort the lipid bilayer, and form a pore. This implies that monomeric membrane-inserted BAX is the key functional unit responsible for initiating mitochondrial outer membrane pore formation and destabilization, leading to apoptosis and cell death. This study provides a new set of tools and previously not accessible data to gain new understanding of the molecular basis of mitochrondrial permeabilization.

107-Plat
Voltage- and Calcium-Dependent Toxin Translocation Across a Tethered Lipid Bilayer
Joel Chopineau[1,2], Remi Veneziano[1], Claire Rossi[3], Jean-Marie Devoisselle[1], Alexandre Chenal[4], Daniel Ladant[4].
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We report the design of novel biomimetic membrane model and its use to characterize *in vitro* the translocation process of bacterial toxin, the adenylate cyclase (CyaA) from *Bordetella pertussis*.
The membrane was assembled over a calmodulin (CaM) layer and exhibits the fundamental characteristics of a biological membrane separating two *cis* and *trans* compartments. SPR was used to monitor the membrane interaction of the CyaA toxin, while the activation of the catalytic activity of CyaA by the tethered CaM was used as a probe of its translocation across the bilayer. Translocation of the CyaA catalytic domain was found to be strictly dependent upon the presence of calcium, and upon application of a negative trans-membrane potential, in good agreement with prior studies done on eukaryotic cells. These results demonstrate that CyaA does not require any eukaryotic components to translocate across a membrane, and suggest that CyaA is electrophoretically transported across the bilayer by the transmembrane electrical field. To our knowledge, this work constitutes the first *in vitro* demonstration of protein translocation across a tethered lipid bilayer. This biomimetic assembly opens new opportunities to explore the molecular mechanisms of protein translocation across biological membranes.

Platform: Biosensors

108-Plat
Electrostatically Enhanced Association of a Pim Kinase Substrate Revealed by Stochastic Detection
Leon Harrington[1], Stephen Cheley[1], Leila T. Alexander[2], Stefan Knapp[2], Hagan Bayley[1].
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A wide variety of analytes may be detected stochastically by observing the modulation of ionic current flowing through a single engineered protein pore. Rate and equilibrium constants for the interaction of an analyte with a binding site are obtained without a need for labels, such as fluorescent tags. We engineered alpha-hemolysin pores containing peptide sensor elements within a single *trans*-side beta-barrel loop. Site-specific proteolysis produced pores bearing peptides tethered by a single N-terminal peptide bond. These pores were used for the single-molecule detection and study of kinase-peptide interactions. Kinetics and affinities of the Pim kinases (Pim-1, Pim-2, Pim-3) for their consensus substrate Pimtide, and of cAMP-dependent protein kinase for an inhibitory peptide, were found to be in good agreement with previously reported values. Distinct current noise behaviors were observed between kinases while bound, which form an additional basis for analyte discrimination. Pim kinases exhibited unusually high association rate constants for their consensus substrate (~10^7-10^8 M^{-1} s^{-1}), extrapolated to zero applied membrane potential. We found this to be due to electrostatic enhancement, which *in vivo* may constitute an additional layer of control between cell signaling pathways.

109-Plat
Probing DNA Methylation in Breast Cancer Cell Lines Using Solid-State Nanopores
Azadeh Bahrami[1], Eric W. Lam[2], Tim Albrecht[1].
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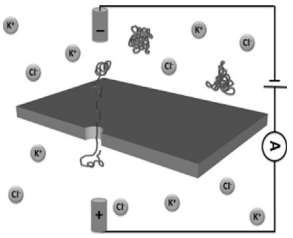
Forkhead box (FOX) transcription factors, including FOXA1, play critical roles in cell proliferation and act as either tumour suppressors or oncogenes. Epigenetic modification of the FOXA1 gene such as DNA methylation has generated considerable interest as a biomarker for monitoring of breast cancer development. Here, we utilised solid-state nanopores as fast, robust and inexpensive biosensor to characterise FOXA1 methylation. The basic principle is the electrophoretically driven passage of individual molecules through a nanopore, which results in detectable changes in the ionic pore current. Figure below illustrates a linear fashion translocation of a negatively charged analyte such as DNA. As soon as the DNA enters the pore, there is a significant reduction in ionic current as part of the liquid-volume is displaced.



In this study, using molecular biology assays, the significance of FOXA1 methylation in various breast cancer cell-lines is demonstrated. Subsequently, in vitro methylated FOXA1 promoter is sensed at single molecule level with a SiNx nanopore, where the detection is enhanced by forming a complex with 5-methylcytosine antibody. Statistical analysis of the translocation process provided us with valuable insight on characteristics and level of methylation on a DNA segment.

110-Plat
Label-Free Detection of the P53-DNA Complex
Philippa Nuttall[1], Eric Lam[2], Tim Albrecht[1].
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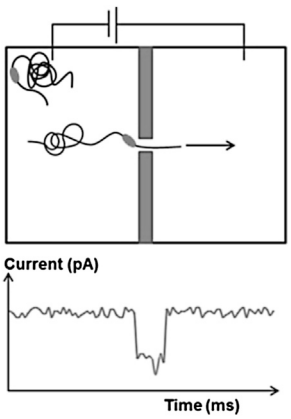
The tumour suppressor p53 plays a crucial role in cell cycle regulation in response to cellular stress such as DNA damage. p53 has been found to be mutated in 50% of all human cancers, the majority of which occur in the DNA binding core domain of the protein. These mutations result in loss of sequence specific binding abilities that activate the transcription of genes to regulate the cell cycle and prevent cell damage proliferation. It is, therefore, crucial to understand the p53-DNA binding interaction to further our ability to detect and combat any breakdown in wild type function.



In this research we investigate the interaction of p53 and sequence specific DNA with label-free techniques. The solid-state nanopore platform is a label-free biosensor that utilises ion transport to drive analytes through the nanopore aperture. The result is a current-time signal that is characteristic to the charge, shape and size of the analyte of interest (see figure). This method allows for detection and differentiation between analytes without a need for labels.
The use of this platform to study the p53-DNA specific binding interaction is combined with AFM analysis to examine binding statistics.

111-Plat
Thermodynamic Characterization of Proteins with Electrically Actuated DNA Nanolevers
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switchSENSE is a novel chip-based bioanalytical method that employs electrically switchable DNA nanolevers as molecular probes. While the nanolevers are driven to oscillate on the surface of microelectrodes by alternating electric fields, their orientation-switching is detected by time-resolved single photon counting. By measuring the molecular dynamics of the switching process it is possible to gauge the hydrodynamic properties of analyte molecules which are bound to the oscillating nanolevers. In particular, changes in the structure or shape of the analyte that alter its hydrodynamic friction coefficient can be monitored with high sensitivity.
Here, we used the switchSENSE approach to investigate the thermally induced unfolding of proteins on a chip. We demonstrate how melting curves can be obtained from molecular dynamics measurements and elucidate the thermal